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Abstract

Both 3-farnesyl salicylic acid and 3-geranyl salicylic acid were synthesized from 2,6-dibromophenol and showed low levels of antimicrobial activity against *E. coli* strains.

Keywords

Antibiotics, Salicylic acid, Synthesis

Disciplines

Organic Chemistry | Veterinary Microbiology and Immunobiology

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Synthesis of 3-Farnesyl Salicylic Acid, a Novel Antimicrobial from *Piper multiplinervium*

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Both 3-farnesyl salicylic acid and 3-geranyl salicylic acid were synthesized from 2,6-dibromophenol and showed low levels of antimicrobial activity against *E. coli* strains.

Keywords: Antibiotics, Salicylic acid, Synthesis.

Of the major classes of antibiotics in use today, only the oxazolidinones and the lipopeptides were introduced after 2000 [1,2]. Both of these antibiotic classes are used against Gram-positive bacterial infections. The recent report by Gupta and coworkers that 3-farnesyl-2-hydroxybenzoic acid (**1**), isolated from the leaves of *Piper multiplinervium* C. DC. (Piperaceae), showed anti-*Helicobacter pylori* activity (MIC 37.5 mg/mL) and antimicrobial activity at MICs in the µg/mL range against *Staphylococcus aureus*, *Escherichia coli*, and other Gram-negative bacteria prompted us to develop a synthesis of members of this class of compounds [3]. Interestingly, with its salicylic acid subunit, these compounds might also exhibit anti-inflammatory activity. Related natural products include *O*-methylgrifolic acid (**2**) that was isolated from the lipophilic fraction of fresh *P. dispanssus*, and grifolic acid (**3**) that was isolated from an American *Albatrellus* species [4].

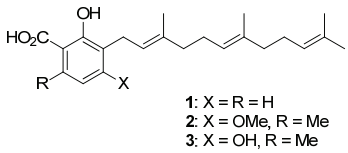
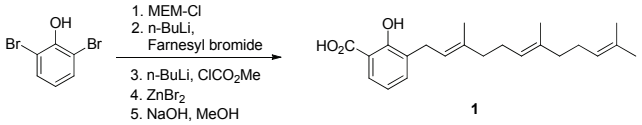


Figure 1: Natural products.

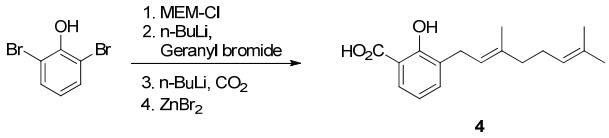
Salicylic acids substituted in the 3-position have been prepared by Mannich condensations [5)] and by acylation [6]. Lau reported a useful synthesis of 3-alkyl salicylic acids in 2001 using a MOM-protected phenol [7]. We prepared the MOM ether of 2,6-dibromophenol. Alkylation of the anion generated from halogen-metal exchange with farnesyl bromide followed by another halogen-metal exchange and reaction with carbon dioxide produced the protected salicylic acid. Unfortunately, the deprotection of the MOM group with aqueous acid gave a complex mixture, presumably due to reactions of the trisubstituted alkenes.

To circumvent this difficulty, we generated the MEM ether of 2,6-dibromophenol in 95% yield by the method of Corey, as shown in Scheme 1 [8]. Halogen-metal exchange, followed by reaction with farnesyl bromide [9], afforded the alkylated compound in 67% yield. Halogen-metal exchange and carboxylation using gaseous carbon dioxide, followed by deprotection of the MEM ether with zinc bromide gave the natural product **1**, albeit in only 10% yield over the last two steps. Substitution of methyl chloroformate for the carbon dioxide gave the ester in 80% yield. Deprotection of the MEM ether and base-mediated hydrolysis of the ester afforded **1** in 50% yield over two steps. This represents the first synthesis of **1**.



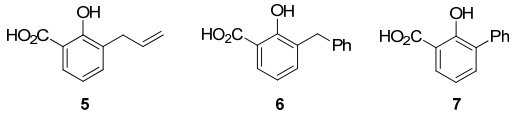
Scheme 1: Synthesis of **1**.

The successful synthesis of **1** prompted us to prepare the geranyl analog **4**. Scheme 2 shows the synthesis of geranyl salicylic acid (**4**) from 2,6-dibromophenol and geranyl bromide [10] in 58% overall yield.



Scheme 2: Synthesis of **4**.

Compounds **1** and **4**, 3-allylsalicylic acid (**5**), prepared from salicylic acid [11], 3-benzylsalicylic acid (**6**), prepared from ortho-benzylphenol using Reimer-Tiemann reaction followed by oxidation [12], and commercially available 3-phenylsalicylic acid (**7**), were tested against two strains of bacteria to determine their level of antimicrobial activity. We used simple zone of inhibition assays in which compounds **1** and **4**, 3-methylsalicylic acid and 3-benzyl salicylic acid (~50 mg/mL), along with solvent (DMSO) alone, were applied to a 10 mm filter paper disc and positioned at the center of an agar plate that had been inoculated with wild type *E. coli* (strain K12). Following incubation at 37°C for 24 h, the zone of inhibition (ZOI) was measured. We observed that 3-methylsalicylic acid, as well as solvent alone, failed to inhibit bacterial growth (ZOI= 0).



5 6 7

Figure 2: Analogs

The synthesis of salicylic acid **1** and analog **4** in four steps from commercially available 2,6-dibromophenol makes available a novel antibiotic for further biological evaluation. Evaluation of 3-methyl salicylic acid and **1** and **4** showed that the alkene is important for biological activity. Zone of inhibition assays yielded the following results. Strain MG1655, salicylic acid and DMSO solvent control: 5 mm, compound **1**: 5.5 mm, and compound **4**: 7 mm; strain NR688, salicylic acid and DMSO: 5 mm, compound **1**: 8 mm, and compound **4**: 14 mm. In contrast, the antibiotic tetracycline gave zones of inhibition of 10 mm (MG1655) and 12 mm (NR688). While compounds **1** and **4** showed low levels of antimicrobial activity against both *E. coli* strains, these results indicate that the antimicrobial activity reported by Rüegg *et al*. [3] cannot be explained solely by the presence of salicylic-acid derivatives.

Experimental

Antimicrobial activity assays: We tested the antimicrobial activity of the compound using a standard disc diffusion assay. For this we inoculated 10 cm LB agar plates with overnight cultures of wild-type *Escherichia coli* K-12 along with a K-12 mutant (strain NR688) with impaired LPS biosynthesis showing heightened sensitivity to hydrophobic drugs [13]. Sterile filter paper disks (5 mm) were positioned at the center of the plates and impregnated with either 2 mg of each compound, or antibiotic control (tetracycline, 15 mg). The diameter of the zone of inhibition of growth around each disk was recorded in mm after overnight incubation at 37°C.

3-Farnesyl salicylic acid (1): In an oven-dried flask under argon, 2,6-dibromophenol (1.0 mmol) was dissolved in 10 mL dry dichloromethane. To this were added diisopropylethylamine (5.0 mmol) and MEMCl (3.1 mmol). The mixture was stirred at room temperature overnight, after which it was worked up with saturated $NaHCO_3$ (5 mL), extracted with dichloromethane, and dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (10% ethyl acetate in hexanes) yielded the protected phenol in 95% yield.
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46 (d, *J* = 7.8 Hz, 2H), 6.82 (t, *J* = 7.95 Hz, 1H), 5.23 (s, 2H), 4.07 (t, *J* = 4.65 Hz, 2H), 3.59 (t, *J* = 4.65 Hz, 2H), 3.35 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 151.7, 133.1, 126.8, 118.8, 98.6, 71.9, 70.1, 59.3.
HRMS (EI) *m/z*: exact mass calcd for $C_{10}H_{12}Br_2O_3$ 337.9153, found 337.9160.

The MEM protected phenol (1.0 mmol) was dissolved in dry THF (10 mL) under argon at -78°C. To this was carefully added a solution of *n*-BuLi (1.0 mmol) and the reaction stirred at -78°C for 30 min. The reaction mixture was treated with a solution of freshly prepared farnesyl bromide (1.0 mmol) in 5 mL THF. The reaction was allowed to warm to rt where it was stirred overnight. The reaction was worked-up with aqueous NH_4Cl, extracted with diethyl ether, and dried over $MgSO_4$ and concentrated *in vacuo*. Purification via silica gel chromatography (5% ethyl acetate in hexanes) yielded the desired compound in 67% yield.
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38 (d, *J* = 6.0 Hz, 1H), 7.12 (d, *J* = 5.7 Hz, 1H), 6.92 (t, *J* = 5.85 Hz, 1H), 5.31-5.28 (m, 1H), 5.18 (s, 2H), 5.14-5.07 (m, 2H), 4.01 (t, *J* = 3.5 Hz, 2H), 3.61 (t, *J* = 3.6 Hz, 2H), 3.46 (d, *J* = 5.4 Hz, 2H), 3.40 (s, 3H), 2.13-1.95 (m, 8H), 1.69 (s, 3H) 1.67 (s, 3H), 1.60 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 152.6, 137.6, 135.4, 133.6, 131.3, 129.3, 128.7, 125.9, 124.5, 123.3, 118.4, 116.4, 98.8, 71.9, 69.7, 59.3, 39.9, 39.5, 29.0, 27.0, 26.7, 26.0, 18.0, 16.4, 16.3.
HRMS (EI) *m/z*: exact mass calcd for $C_{25}H_{37}BrO_3$ 464.1926, found 464.1938.

The farnesyl adduct (0.67 mmol) was dissolved in 10 mL dry THF at -78°C, where it was treated with *n*-BuLi (0.68 mmol) for 30 min. A solution of methyl chloroformate (1.0 mmol) in THF was added to the reaction mixture, which was then warmed to rt and allowed to stir overnight, after which the reaction was quenched with HCl. The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated *in vacuo*. Purification via silica gel chromatography (15% ethyl acetate in hexanes) yielded the pure compound in 80 % yield.
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (dd, *J* = 7.8 Hz, 1.8 Hz, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.07 (t, *J* = 7.65 Hz, 1H), 5.32-5.27 (m, 1H), 5.13 (s, 2H), 5.13-5.05 (m, 2H), 3.92 (t, *J* = 4.65 Hz, 2H), 3.87 (s, 3H), 3.57 (t, *J* = 4.65 Hz, 2H), 3.46 (d, *J* = 7.2 Hz, 2H), 3.37 (s, 3H), 2.12-1.96 (m, 8H), 1.67 (s, 3H), 1.66 (s, 3H), 1.58 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 167.0, 155.7, 137.3, 136.7, 135.5, 134.1, 132.6, 129.4, 125.1, 124.6, 124.2, 122.2, 100.3, 71.9, 69.4, 59.3, 52.3, 39.9, 32.2, 28.3, 26.9, 25.9, 23.6, 17.9, 16.4, 16.3.
HRMS (EI) *m/z*: exact mass calcd for $C_{27}H_{41}O_5$ 445.2949, found 445.2954.

The MEM protecting group was removed using $ZnBr_2$. The $ZnBr_2$ was freshly prepared by suspending oven-dried zinc powder (7.3 mmol) in 10 mL dry THF. To this was added 1,2-dibromoethane (8.1 mmol) and the solution was heated to reflux overnight, during which time the color turned cloudy white. Methyl ester from the previous step (1 mmol) was added to the freshly prepared $ZnBr_2$ solution. The reaction mixture was stirred overnight at room temperature. The reaction was worked up with H_2O, extracted with diethyl ether, dried over $MgSO_4$ and concentrated *in vacuo*. Purification via silica gel chromatography (5% ethyl acetate in hexanes) yielded pure compound in 70% yield.
^{1}H NMR (300 MHz, $CDCl_3$): δ 11.05 (s, OH), 7.69 (d, *J* = 8.1 Hz, 1H), 7.32 (d, *J* = 7.5 Hz, 1H), 6.80 (t, *J* = 7.65 Hz, 1H), 5.36-5.31 (m, 1H), 5.14-5.07 (m, 2H), 3.94 (s, 3H), 3.38 (d, *J* = 7.5 Hz, 2H), 2.12-1.99 (m, 8H), 1.70 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$): 171.3, 159.9, 137.2, 135.4, 130.2, 127.6, 125.2, 124.6, 124.3, 121.8, 118.8, 111.9, 52.4, 39.9, 32.2, 27.8, 26.9, 26.7, 26.6, 26.0, 17.9, 16.3.
HRMS (EI) *m/z*: exact mass calcd for $C_{23}H_{33}O_3$ 357.2424, found 357.2430.

To a solution of the phenolic compound (1 mmol) from the previous step in MeOH (5 mL) was added a solution of NaOH (4 mmol) in H_2O (6 mL) and the resulting suspension was heated at 55°C for 3 h. After the reaction was complete, it was cooled to room temperature and washed with diethyl ether. The aqueous layer was acidified with 1N HCl and the suspension extracted with ethyl acetate, dried over $MgSO_4$ and concentrated *in vacuo* to give the farnesyl salicylic acid (**1**) in 72% yield.
^{1}H NMR (300 MHz, $CDCl_3$): δ 10.68 (s, OH), 7.79 (d, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 7.2 Hz, 1H), 6.86 (t, *J* = 7.65Hz, 1H), 5.36-5.32 (m, 1H), 5.15-5.07 (m, 2H), 3.39 (d, *J* = 7.5 Hz, 2H), 2.15-1.99 (m, 8H), 1.72 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 175.4, 160.6, 137.3, 136.6, 135.3, 130.5, 128.7, 125.2, 124.6, 124.3, 121.6, 119.2, 110.8, 40.2, 40.0, 32.2, 27.9, 26.7, 25.9, 17.9, 16.4, 16.3.

HRMS (EI) *m/z*: exact mass calcd for $C_{22}H_{29}O_3$ 341.2122, found 341.2129.

3-Geranyl salicylic acid (4): The MEM-protected phenol (0.93 mmol) in THF (10 mL) was treated with 2.5M *n*-BuLi (0.93 mmol) at -78°C for 30 min under argon. The resultant mixture was treated with a solution of freshly prepared geranyl bromide (1.1 mmol) in THF (5 mL). The reaction mixture was allowed to warm to rt where it was stirred overnight. The reaction was worked up with aqueous NH_4Cl, extracted with diethyl ether, dried over $MgSO_4$ and concentrated *in vacuo*. Purification via silica gel chromatography (5% ethyl acetate in hexanes) yielded the geranyl adduct in 83% yield.
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 (d, *J* = 6.0 Hz, 1H), 7.11 (d, *J* = 6.0 Hz, 1 H), 6.93 (t, *J* = 6.0 Hz, 1H), 5.28 (t, *J* = 6.0 Hz, 1H), 5.18 (s, 2H), 5.10 (t, *J* = 6.0 Hz, 1H), 4.02 (t, *J* = 6.0 Hz, 2H), 3.62 (t, *J* = 6.0 Hz, 2H), 3.46 (d, *J* = 6.0 Hz, 1H), 3.40 (s, 3H), 2.10-2.05 (m, 4H),1.68 (s, 6H), 1.60 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 152.6, 137.3, 133.6, 131.8, 131.3, 129.3, 125.9, 124.4, 122.2, 117.6, 98.8, 71.9, 69.7, 59.3, 39.9, 29.0, 26.8, 26.0, 18.0, 16.4.
HRMS (EI) *m/z*: exact mass calcd for $C_{20}H_{29}BrO_3$ 396.1300, found 396.1308.

Geranyl adduct (0.77 mmol) was dissolved in 10 mL dry THF at -78°C, where it was treated with 2.5M *n*-BuLi (0.78 mmol) for 30 min. Carbon dioxide gas was bubbled through the solution and warmed to room temp for 2 h. The reaction was worked up with acetic acid and concentrated. The crude product was used in the next step without further purification.

Zinc bromide was prepared on the same scale as in the synthesis of **1**. The starting material was added to the zinc bromide solution in THF and stirred overnight. The reaction was worked up with H_2O, extracted with diethyl ether, dried over $MgSO_4$ and concentrated *in vacuo*. Purification via silica gel chromatography (15% ethyl acetate in hexanes) yielded **4** as an off-white solid in 74% yield over 2 steps.
^{1}H NMR (300 MHz, $CDCl_3$): δ 10.72 (br, OH), 8.02 (d, *J* = 6.0 Hz, 1H), 7.38 (d, *J* = 6.0 Hz), 7.03 (t, *J* = 6.0, 1H), 5.32 (t, *J* = 6.0 Hz,1H), 5.10 (t, *J* = 6.0 Hz, 1H), 3.51 (d, *J* = 6.0 Hz, 1H), 2.03-1.96 (m, 4H), 1.68 (s, 6H), 1.60 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 153.1, 136.8, 136.0, 132.0, 131.2, 124.2, 121.6, 119.7, 116.0, 47.8, 41.2, 39.8, 32.0, 23.1, 19.9.
HRMS (EI) *m/z*: exact mass calcd for $C_{17}H_{22}O_3$ 274.1569, found 274.1575.

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